Functional Genomics Core -Analysis of Stress Response Pathways in Metal-Reducing Bacteria

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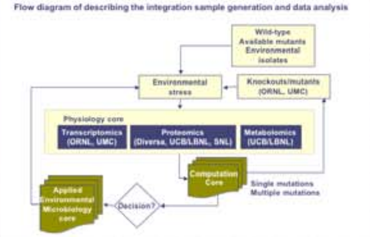
Virtual Institute of Microbial Stress and Survival

Introduction

Environmental contamination by metals and radionuclides constitutes a serious problem in many ecosystems. Bioremediation schemes involving dissimilatory metal ion-reducing bacteria are attractive for their cost-effectiveness and limited physical detriment and disturbance on the environment. *Desulfovibrio vulgaris*, *Shewanella oneidensis*, and *Geobacter metallireducens* represent three different groups of organisms capable of metal and radionuclide reduction whose complete genome sequences were determined under the support of DOE-funded projects. Utilizing the available genome sequence information, we have focused our efforts on the experimental analysis of various stress response pathways in *D. vulgaris* Hildenborough, using a repertoire of functional genomic tools and mutational analysis

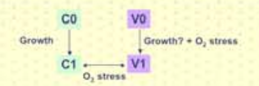
Flow diagram of describing the integration sample generation and data analysis

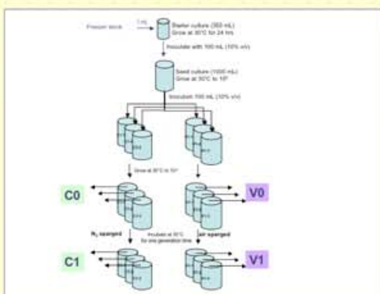


D. vulgaris O_2 stress response:

Experimental design

Description of the experimental design for the analysis of the stress response of *D. vulgaris* exposed to air for up to 5 hrs (~ 1 doubling time) compared to cells exposed to N_2. Samples at time 0 (CO and V0) and time 5 hrs (C1 and V1) were pooled for the proteomics analysis. Samples for microarray analysis were taken at 2 hrs.





O_2 stress gene expression

The whole genome oligo-based microarray covers all ORFs in the genome with 3574 oligos, including 3471 (97.1%) unique probes and 103 (2.9%) probes which may cross-hybridize with other ORFs

Down-Regulated Genes

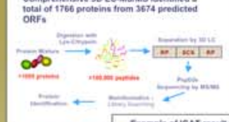
Description	logRatio	Z	VIMSS#
[illegible]	[illegible]	[illegible]	[illegible]

Up-Regulated Genes

Description	logRatio	Z	VIMSS#
ATP-dependent RNA helicase DeaD (deaD)	3.27	6.13	208836
histidyl-tRNA synthetase (hisS)	2.50	4.66	208895
GTP-binding protein TypA (typA)	2.74	4.66	207715
ribose-phosphate pyrophosphokinase (prsA)	2.29	4.19	207020
ribosomal protein L3 (rplC)	2.19	4.17	206745
peptide chain release factor 1 (prfA)	2.15	4.14	208424
ribosomal protein L36 (rpmJ)	2.17	4.03	206767
cytidine/deoxycytidylate deaminase family protein	2.11	3.87	206641
ribonuclease P protein component (rnpA)	2.66	3.85	208510
ATP-dependent RNA helicase DeaD (deaD)	2.06	3.51	208604
3-oxoacyl-(acyl-carrier-protein) synthase III (fabH)	1.94	3.50	206646
DNA-directed RNA polymerase, alpha subunit (rpoA)	1.79	3.44	206771
MinD-related protein	1.72	3.34	207329
amino acid ABC transporter, permease protein (opuBB)	1.99	3.30	207795
ribosomal protein L14 (rplP)	1.73	3.29	206752
methionine aminopeptidase, type I (map)	1.67	3.26	206766
mrp protein (mrp)	1.76	3.25	207585
lipoic acid synthetase (lipA)	2.00	3.21	206333
glycine betaine/L-proline transport ATP binding subunit (proV)	1.76	3.20	207796
RecF protein, putative	1.71	3.20	207497
HlyD family secretion protein (acrA)	1.93	3.10	208327
Helix-turn-helix protein, copG family domain protein	1.72	3.09	208400
inner membrane protein, 60 kDa (yidC)	2.14	3.08	206512
[illegible]	[illegible]	[illegible]	[illegible]

O_2 stress protein expression

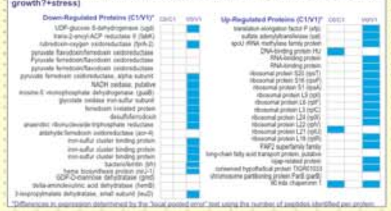
Comprehensive 3D LC-MS/MS identified a total of 1766 proteins from 3674 predicted ORFs



DIGE Cy3/Cy5 overlay of C1 vs V1 samples

Example of ICAT result for one heavy:light pair

O_2 stress induced proteins (C1 vs V1) as determined by 3D LC MS/MS an comparison to their differential expression profiles to C0 vs C1 (effect of growth) and V0 vs V1 (effect of growth?+stress)



Comparison of O_2 stress genomic data

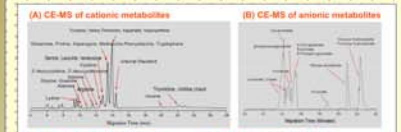
List of the differentially expressed proteins identified by any of the three techniques and their corresponding gene expression

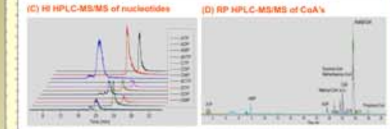
	Microarray		Proteomics		
	Log ratio	modified Z score	Comprehensive	DIGE*	ICAT*
[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]

Analysis of metabolites

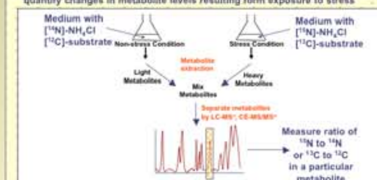
Methods were developed to quantify as many metabolites from *Desulfovibrio vulgaris* as possible. A CE-MS equipped with a either a bare fused-silica capillary column for cationic metabolites (A) or a polymer coated SMILE (+) capillary column for anionic metabolites (B). Companion HPLC-MS/MS methods were also developed analyzed nucleotides on a hydrophilic interaction (HI) column (C) and CoA's on a C_{18} reverse phase (RP) column (D).

By a combination of these techniques, we can now analyze over 100 metabolites from cell extracts





We are currently developing differential metabolome analysis methods to quantify changes in metabolite levels resulting form exposure to stress



Summary

- Gene expression analysis using
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Sulfate reducing pathway showing the three enzymes found to be down regulated upon exposure to oxygen